MEDICAL AND ENVIRONMENTAL EFFECTS OF UV RADIATION

B. M. SUTHERLAND
Brookhaven National Laboratory
Upton, NY, 11973, USA

1. Introduction

Organisms living on the earth are exposed to solar radiation, including its ultraviolet (UV) components (for general reviews, the reader is referred to Smith [1] and Young et al. [2]). UV wavelength regions present in sunlight are frequently designated as UVB (290-320 nm) and UVA (320-400 nm). In today's solar spectrum, UVA is the principal UV component, with UVB present at much lower levels. Ozone depletion will increase the levels of UVB reaching the biosphere, but the levels of UVA will not be changed significantly [3]. Because of the high efficiency of UVB in producing damage in biological organisms in the laboratory experiments, it has sometimes been assumed that UVA has little or no adverse biological effects. However, accumulating data [4, 5], including action spectra (efficiency of biological damage as a function of wavelength of radiation; see Section 5) for DNA damage in alfalfa seedlings [6], in human skin [7], and for a variety of plant damages (Caldwell, this volume) indicate that UVA can induce damage in DNA in higher organisms. Thus, understanding the differential effects of UVA and UVB wavebands is essential for estimating the biological consequences of stratospheric ozone depletion.

2. Principles of Light Absorption

Underlying all the biological effects of UV radiation is the absorption of the UV

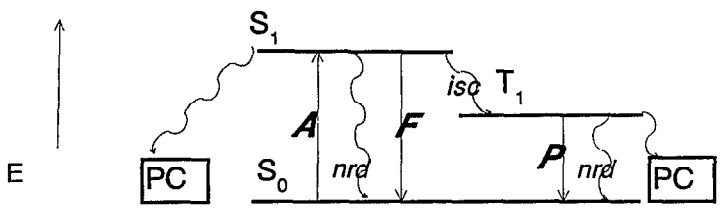

Figure 1. Energy level diagram showing light absorption, radiative and non-radiative emissions and photochemical reactions.

photon. Figure 1 shows an energy level diagram, with the ground singlet state (S_0), lowest excited singlet state (S_1) and lowest excited triplet state (T_1). In this diagram, the vertical axis corresponds to increasing energy.

In absorption, a photon of at least the same energy spacing as the S_0-S_1 energy gap is absorbed (A) by the biomolecule, raising the molecule to the lowest excited singlet. The energy may be transferred non-radiatively (shown by curved lines; by rotation, translation, etc, without photon emission) to the T_1; this is termed intersystem crossing (isc). Both the S_1 and T_1 may relax by non-radiative emission (nre) to the ground state. The energy may also be emitted promptly (within picoseconds to nanoseconds) from the S_1 by emission of a photon, i.e., producing fluorescence (F). If the molecule has undergone intersystem crossing to the lowest excited triplet, it may emit energy via photon emission, termed phosphorescence (P). Since the latter process involves a change in spin state, it is a "forbidden" transition, and thus may be significantly delayed—from microseconds to as much as seconds, minutes, or even hours—with respect to the original absorption event.

The excited molecule may dissipate the absorbed energy by undergoing photochemistry (PC). Photochemical reactions may occur from either the S_1 or the T_1. In some molecules different reactions may result from photochemistry from specific excited states. The occurrence of photochemistry in an excited molecule competes with but does not preclude energy dissipation by light emission and by non-radiative decay.

This brief summary of photon absorption and energy dissipation points out two important points: first, only a photon with the same energy as the energy difference that matches or exceeds the gap between S_0 and S_1 will be absorbed. (For this simplified discussion, vibrational and rotational states of molecules are ignored.) For a more complete discussion, the reader is referred to classic photochemistry texts such as Calvert and Pitts [8]). This is known as the First Law of Photochemistry, which is sometimes stated as "No photochemical reaction without light absorption." Second, light absorption occurs by quantum events, i.e., by absorption of discreet photons, not of an energy continuum. This is critical in analysis of action spectra, in which we often want to compare an action spectrum (See Section V) with the absorption spectra of candidate target molecules.

3. Potentially Important Target Biomolecules

UV radiation reaching the surface of the earth includes wavelengths in the range 290-400 nm. Since the First Law of Photochemistry (see above) tells us that a photon must be absorbed for photochemistry to occur, we can ask which biomolecules absorb radiation in this wavelength range. Proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) all absorb in range 290-400 nm.

Many studies show that proteins can be inactivated by UV radiation (for an excellent summary, see Setlow and Pollard [9]). Since absorption must precede photochemistry, amino acids with substantial molar extinction coefficients (ε) for UV absorption would be good candidates for absorbing photons in the ultraviolet range that could lead to protein inactivation by UV radiation. The aromatic amino acids, tryptophan and tyrosine, indeed have εs in the range of 1000-10,000 at ~ 280 nm. However, they have rather low quantum yields (~ 0.005) for photochemical reactions. Nonetheless, proteins with aromatic amino acids (but little cystine, see below) are

inactivated by UV and their inactivation action spectrum resembles their absorption spectrum. Cystine is an important target for protein inactivation. Although its molar extinction coefficient is only ~ 100 at 280 nm, its high quantum yield (0.05) and critical role in maintaining protein three-dimensional structure make it the most sensitive residue in producing protein inactivation. Relative to nucleic acids, most proteins are resistant to inactivation by UV. A major source of this resistance is the much lower absorption cross section of proteins, about one-twentieth of that of nucleic acids at ~ 2700 nm. Although some data suggest that Photosystem II of the photosynthetic apparatus is photolabile [10, 11], current data suggest that the effect may not be biologically significant (see Nogues, this volume). RNA is also susceptible to UV damage, forming pyrimidine hydrates as well as pyrimidine photodimers.

Photoproducts are also induced in DNA by UV radiation. Figure 2 shows the induction of a cyclobutane pyrimidine dimers in DNA. UV also induces [6-4] pyrimidine-pyrimidone photoproducts, plus other minority products, principally photoproducts of pyrimidine bases.

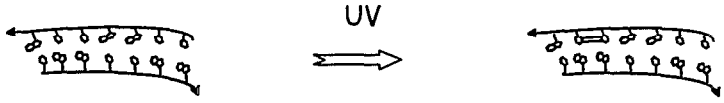

Figure 2. Induction by UV of a cyclobutyl pyrimidine dimer in DNA

The cyclobutyl pyrimidine dimer (CPD) is the numerically predominant class of UV photolesion in DNA. The 6-4 photoadduct is induced at about 10-30 % of the level of CPD, and other photoproducts are formed much lower levels.

Other factors that must be considered are the cellular numerology, and importance of function of the specific molecule. Numerology involves the number of the specific molecules present in a cell. If a thousand of copies of a protein or RNA are present in a cell, and 10% of them are inactivated by UV, the remaining 900 may be quite adequate for cell function. In addition, protein and RNA turnover are normal in cells, and there are well-developed and coordinated paths for metabolism—including salvage paths—of damaged proteins and of RNA. Further, proteins and RNA can be replaced by de novo synthesis, as long as the DNA and cellular machinery for protein synthesis are intact.

4. Measuring DNA Damage

Many approaches have been developed for measuring DNA damage, including those based on chromatography, immunology, and various properties of undamaged and damaged DNA. In the latter category is gel electrophoresis/electronic imaging/number average length analysis. It is a powerful approach of DNA lesion quantitation that stems from physical chemical methods for characterization of polymers. It provides absolute measurements of lesion frequencies (lesions per kilobase or per megabase or even per gigabase). With appropriate experimental modifications, it can be used to quantify lesion frequencies over some six orders of magnitude. It does not require that the lesions have any specific distribution on the DNA, and, in specific, does not require a random distribution of lesions.

Number average length analysis can be used to measure lesions affecting one DNA strand, in which case the DNA is dispersed according to single strand molecular length using denaturing conditions. For assessment of lesions affecting one strand, in which DNAs are dispersed on denaturing gels, it is essential to use fully single-stranded molecules as molecular length standards to establish DNA dispersion curves. Partially denatured molecules can form branched structures, whose electrophoretic mobility may not be a direct function of the DNA molecular length. In the case of lesion determination on denaturing gels, the lesion frequencies are computed in units of damage are lesions per base, or per kilobase (kb, 10^3 bases), or per megabase (Mb, 10^6 bases), or per gigabase (Gb, 10^9 bases), etc. For determinations on non-denaturing gels, the damage frequencies are computed in terms of kilobase pairs (kbp), megabase pairs (Mbp) or gigabase pairs (Gbp).

This method can also measure damages affecting both DNA strands; in this case, the double strand molecular length standard DNAs are electrophoresed on the same gel as the experimental samples. Again, no specific distribution of damages is required. In this case, the damage frequency is given as damages per base pair, per kilobase pair, or even per gigabase pair. Figure 3 shows the principles of damage

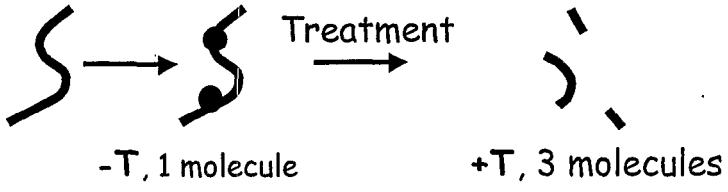

Figure 3. Induction of lesions in a DNA molecule and treatment with a lesion-specific agent

measurement by these methods. A DNA molecule is damaged by an agent to produce two lesions shown as solid circles. The population of molecules is divided into two, and one portion is treated with an agent that makes a DNA strand break at the site of each lesion. Some examples of such lesion-specific agents are the T4 Endonuclease V, which—among UV-induced lesions—specifically recognizes cyclobutyl pyrimidine dimers [12]. (More recently it has been shown that T4 Endo V also recognizes FapyAdenines, oxidized bases induced by ionizing radiation and at low frequencies by UV [13].) The other portion is incubated without the lesion-specific treatment. We note that the treated population now consists of three molecules, while there is only one molecule in the untreated population. Simple arithmetic shows that the number of lesions (L, two) can be calculated by subtracting the number of molecules in the untreated population (N_u, one) from the number in the treated population (N_t, three).

$$L = N_t - N_u \tag{1}$$

Generally, however, we want to know the Frequency (F) of damages, i.e., lesions per base or per thousand bases or per million bases. This is readily computed by dividing each term by the number of bases (b) in the treated and untreated samples, i.e.

$$F = L/b = N_t/b_t - N_u/b_u. \quad (2)$$

Since each term is divided by the quantity of DNA in that sample, the result does not depend on the mass of DNA in each sample, i.e., the procedure is "self-normalizing." Thus the problem of measuring damage levels becomes one of counting the number of molecules in each DNA sample before and after treatment with the lesion-specific agent. One method for "counting" DNA molecules is to separate them according to molecular size by agarose gel electrophoresis, and then to measure the quantity of DNA molecules of each size, computed from a DNA dispersion curve established by molecular length standard DNAs electrophoresed on the same agarose gel [14]. An electronic image is obtained of an electrophoretic gel containing sample DNAs stained with ethidium bromide. A DNA dispersion function is derived from the molecular length standards in the gel. The size distribution of molecules (or number average molecular length, which is the ordinary average of the size of each molecule multiplied by the number of molecules in each size class) in each sample is calculated from the relation

$$L_n = \frac{\int f(x)\ dx}{\int \frac{f(x)}{L(x)} dx}, \quad (3)$$

where $L(x)$ is the length of the DNA molecules that migrated to position x, and $f(x)dx$ is the intensity of ethidium fluorescence from DNA molecules at that position.

From the L_ns of the treated and untreated populations, the frequency of lesions, ϕ_D, is computed from

$$\phi_D = 1/L_n\,(+\ treatment) - 1/L_n\,(-\ treatment), \quad (4)$$

where $1/L_n(+\ treatment)$, and $1/L_n(-\ treatment)$, are the reciprocals of the L_ns of samples that were treated or not treated, respectively.

Since this method measures the lesion frequency per molecule, the sensitivity of lesion frequency measurement can be increased substantially by increasing the size of the molecules examined. In practical terms this means that improved methods for isolating larger and larger DNA molecules are required. For damages affecting both strands, this means that the level of double strand breaks induced in DNA isolation must be minimized. However, for lesions affecting only one DNA strand, this requires that the level of single strand breaks induced during isolation must be minimized, a much more rigorous requirement. Isolation of DNA by methods that avoid chemical extraction, including enzymatic digestion while the DNA is stabilized mechanically within an agarose "plug" or "button," provides a successful approach to meeting such requirements [15-17].

With high sensitivity methods of detection (quantitative detection by charge coupled device imaging [18-20] of fluorescence from a DNA-bound molecule such as ethidium bromide, whose fluorescence is directly proportional to the mass of DNA over

several orders of magnitude, very small quantities of DNA are required for a measurement, and lesions can be measured at very low frequencies. For a homogeneous DNA, i.e., a molecular species of one size and conformation, less than one ng DNA can be used. For damage determination in higher organisms, which contain heterodisperse DNAs, about 50 ng of DNA are used for routine assays [20, 21]. The sensitivity of number average length determination depends on two factors: the size of the DNA molecules and the minimum fraction of molecules that must be broken to be detected in the specific combination of biochemical, electrophoretic, imaging system and computer analysis that is used. For double-strand DNA populations in the size range of ~1 Mbp, damages can be measured down to ~ 5/Gpb [22].

A wide variety of damages can be quantitated by this approach. Lesions affecting one DNA strand are measured by treatment with a lesion-specific cleaving agent and dispersion under conditions that separate the DNA strands ("denaturing" conditions), and the resulting frequencies are computed as lesions/base. Damages affecting both strands are measured by treatment with the agent as above, but with dispersion under conditions that do not separate the DNA strands, i.e., "non-denaturing" conditions. Strand breaks, whether single strand or double strand breaks, are measured directly, since they in themselves comprise breaks of the phosphodiester backbone.

In addition, lesion–specific or lesion class–specific enzymes that cleave the phosphodiester backbone at each lesion site can be used to convert each lesion to a strand break. Examples of DNA lesions and the cleavage agents that recognize them include: cyclobutyl pyrimidine dimers, T4 endonuclease V [12] or *Micrococcus luteus* UV endonuclease [23, 24]; oxidized purines *Escherichia coli* Fpg protein [25]; oxidized pyrimidines, *Escherichia coli* Endonuclease III [26]. Many of these enzymes have multiple substrates, which must be taken into account. For example, the glycosylase activity of T4 endonuclease V removes the dimer from DNA, and its lyase activity attacks the resulting abasic site(s) and cleaves the DNA [27]. By its lyase activity, this enzyme cleaves DNA at 'regular' abasic sites, i.e., a base-less sugar moiety resulting from cleavage of the N-glycosyl bond to the base [27]. It also recognizes FapyAdenine residues induced by ionizing radiation and in low yields by UV [13].

5. Action Spectroscopy: Powerful Approach For Understanding UV Effects

Action spectra relate the effectiveness of specific wavelengths in producing an effect.

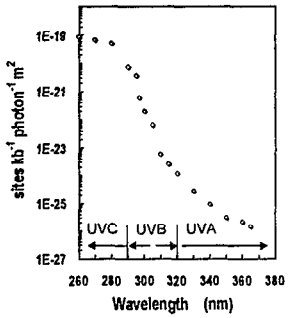

Figure 4. Action spectrum for induction of cyclobutyl pyrimidine dimers in T7 DNA in solution

For analytical action spectra designed to elucidate the absorbing moiety (chromophore) or mechanism of reaction, Jagger has an excellent description of the criteria that must be met for obtaining valid data [28]. These are:

- The quantum yield must be the same at all wavelengths.
- The absorption spectrum of the chromophore must be the same in vivo as in vitro.
- Intracellular screening, such as absorption or scattering within a cell, must be either negligible or constant at all wavelengths.
- The incident radiation is not entirely absorbed by the sample at any wavelength of interest.
- Reciprocity of time and UV exposure holds under the conditions of the determinations.

For DNA in dilute solution and short path length, it is easy to fulfill these criteria. The data in
Figure 4 show an action spectrum for induction of cyclobutyl pyrimidine dimers in purified DNA in dilute solution [29]. The action spectrum in the UVC (220-290 nm) and UVB (290-320 nm) resembles the absorption spectrum of DNA. The high sensitivity of the gel electrophoresis/number average molecular length analysis method, which can range over some 5 or 6 orders of magnitude, allows the quantitation of very low frequencies of dimers induced by UVA (320–400 nm).

Measurement of analytical action spectra in biological systems, especially in higher organisms, is fraught with difficulties. Consideration of the criteria of Jagger [28] outlined above indicates that many are difficult to fulfill in complex systems. Some of these apparent "complications" can be useful in providing critical information

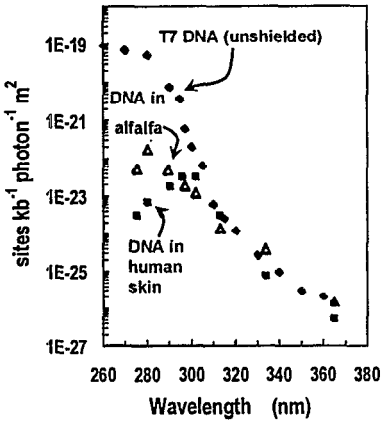

Figure 5. Action spectra for pyrimidine dimer induction in T7 dna in solution, in dna in alfalfa seedlings, and in human skin in situ

on the biological system. For example, the action spectrum in a complex organism for a DNA damage that results from direct photon absorption in DNA provides information on the internal absorption/shielding/scattering that protects DNA from damage by incident photons. Figure 5 shows action spectra for formation of cyclobutyl pyrimidine

dimers in DNA in alfalfa seedlings [6] and in human skin, respectively [7], exposed to narrow band UV radiation. These data are compared with those for T7 DNA in solution (diamonds) [29]. These spectra clearly show that in the shorter UV wavelength region, much lower damage levels are formed in both alfalfa and in human skin than in isolated DNA. Again, the high sensitivity of the number average length analysis method allows the quantitation of dimer induction at long wavelengths. At longer wavelengths, the spectra are rather similar to that of isolated DNA, indicating a measurable but very low level of damage induced by UVA radiation.

The new action spectra for UV- induced damage to plants obtained by Caldwell and his associates (this volume) resemble the alfalfa action spectrum of Quaite et al. [6] (it should be noted that Caldwell's previous 'generalized plant action spectrum' [30-32] differed strikingly from the alfalfa action spectrum, principally because no biological damage data from wavelengths longer than 313 nm was considered in construction of the 'generalized plant action spectrum.')

6. Biological Effects of Long Wavelength Environmental UVA

The action spectra for DNA damage induction discussed in Section 5 clearly show that UVA radiation can induce photoproducts in DNA. Most studies evaluating the effect of ozone depletion have centered on UVB, since conditions of ozone depletion increase the number of photons in this wavelength region reaching the surface of the earth. The predominant source of UV in solar radiation is UVA, and the levels of UVA will not be altered significantly by ozone depletion. However, evaluating the biological effect of increased UV requires that we know the damage induced by UVA under normal (in absence of ozone depletion) solar conditions. That is, if UVA induced no damage, and all DNA damage resulted from the low levels of UVB reaching the biosphere, then a biologically-weighted increase (e.g., 10 %) in UVB would be expected to increase DNA damage by 10%. However, if the high level of UVA in normal solar conditions induces 80% of the total DNA damage, and UVB induces only 20%, an additional 10% UVB damage would result in only ~ 2% increase in total damage.

Current UVA sources limit the accuracy and resolution of action spectra in this critical wavelength range. First, there are few Hg emission lines in this wavelength range: 334, 365, 391 and 398 nm. To obtain radiation from a specific line, a monochromator or narrow band filters are used. It should be noted that Hg-Xe lamps and even Hg lamps have some emission between these lines, and thus it is essential to determine the wavelength distribution of the light emerging from the monochromator and to use appropriate cut-off filters as necessary. It is not valid to assume that the wavelength specified on a dial of a light source is the only wavelength (or wavelength range) emitted by the source, that the wavelength calibration is accurate or that there are no contaminating wavelengths in the beam.

Some UVA wavelengths are available from lasers, but one must be careful that the high peak power does not produce two-photon effects that can result in different photochemical reactions from the one-photon mediated effects from lower intensity sources. Further, some lasers have high brightness (intensity per unit wavelength per unit timer per unit source area per unit solid angle), but do not provide sufficient total photon flux to irradiate a sufficient area for most biological samples. The paucity of

lines (or wavelengths from lasers) in this region thus severely limits the resolution of the spectra that can be obtained.

One possible solution is to use a Hg-Xe lamp, which provides a continuum of radiation between the Hg lines. However, in attempting to obtain higher intensity, the slits of the monochromator may be opened wider, thus increasing the range of wavelengths in the incident beam. The pitfalls in using wider bandwidths to measure action spectra in which the slope of the spectrum is changing rapidly in the wavelength region of interest are discussed by Sliney in this volume. It is critical that scattered light from other wavelength ranges be rigorously excluded. If they are not, the shorter wavelength UV may dominate the observed biological effects, which would then be erroneously perceived as resulting from the UVA.

An additional problem is the lack of intensity in the UVA region available from most sources. Since the cross-section for most biological effects in this wavelength range is small, this results in long exposures (many minutes or even hours) for production of observable damage in many biological systems. In isolated molecules, e.g. DNA in solution, as long as nucleases that could degrade the DNA are rigorously excluded by the use of sterile buffers, the presence of EDTA, etc., long exposures are an inconvenience, but do not affect the scientific result.

However, results from biological systems capable of repair may be significantly affected by extended irradiation: simultaneous repair may reduce damages induced by the UVA radiation so that the actual damage level is underestimated. Such repair could be carried out by light-independent repair processes (nucleotide excision repair or base excision repair). Moreover, cells capable of photoreactivation present even more complex responses. Photoreactivation is a one-enzyme repair path, in which a single enzyme, photolyase, binds to a cyclobutyl pyrimidine dimer in DNA; upon absorption by the photolyase-dimer complex of a photon in the visible or UVA range, the dimer is monomerized and the photolyase is liberated to seek another dimer.

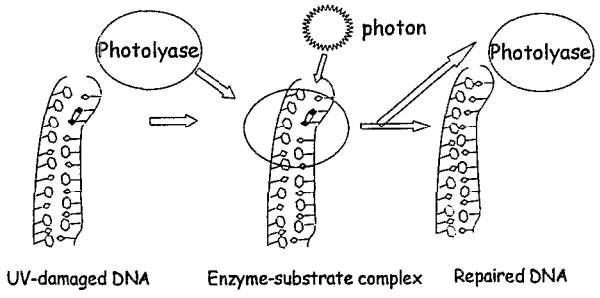

Figure 6. Photolyase repairs cyclobutyl pyrimidine dimers in DNA

The first step—association of enzyme and substrate—is temperature and concentration dependent [33]. However, the second step—dimer photolysis—is a photochemical reaction, and virtually independent of temperature [34]. As a light-driven, enzymatic reaction, the rate of photorepair depends not only on the number of photolyases per cell but also on the wavelength of the light. Further, this enzyme has two substrates: the dimer and the photolyzing photon. Simple biochemical considerations indicate that the rate of photorepair depends on the substrate

concentration, with the maximal rate at a "saturating" concentration of the substrate, i.e., the concentration such that further increases do not increase the reaction rate. Since photolyase has two substrates, the dimer and the photon, the rate of photorepair depends not only on the dimer concentration (frequency) in the cell but also on the wavelengths and intensity of the photoreactivating light.

Thus we can summarize the factors affecting the net effect of UVA as: Net effect of UVA = UVA-induced damage – UVA-mediated repair - Light independent repair.. The UVA-induced damage depends on the wavelengths and intensities at each wavelength and the cross-section of the biological system for damage at each wavelength. The UVA-mediated repair term depends on the number of photolyases in a cell, the temperature, the photorepair action spectrum for that photolyase, the wavelength and intensity of the light, as well as the level of dimers per cell. The light-independent repair is dependent on the genotype of the cell, the dimer frequency, the presence of other damages that are also substrate for the repair system, the level of these enzymes in the cell and on the substrates required for that repair, e.g. ATP, used as an energy source.

Thus, obtaining robust data on the effects of UVA is challenging. Even with careful attention to biological, physical and biochemical factors, current light sources are far from ideal, being limited in intensity and in wavelength. An ideal source for UVA radiation would provide a continuous selection of wavelength, high intensity over a substantial area, and the time structure of the radiation should avoid high peak power/low inter-peak modes that produce multi-photon photochemistry. Free electron lasers in the UV range are being developed at several institutions; at least two of these should provide high time-average power in the UVA range [35]. They may offer powerful new next-generation sources for UVA photochemistry and photobiology. The elucidation of the actual biological role of UVA awaits the development of capability of irradiation of biological systems at such facilities.

Acknowledgments

Research supported by the Office of Biological and Environmental Research of the US Department of Energy.

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